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- b) catalyzing the deposition of tyramide in said cells comprising said intracellular analyte;
- c) contacting said cells with a medium comprising a chaotropic agent to wash said cells;
- d) contacting said cells with a detectable label that directly or indirectly binds to tyramide, whereby cells comprising said intracellular analyte are specifically labeled; and
- e) detecting a signal from cells comprising said detectable label using a flow cytometric device, wherein the presence of said signal is correlated to the presence of said intracellular analyte in said cells.

2. (Amended) A method of detecting the presence of an intracellular analyte in one or more cells by flow cytometry, the method comprising:

- a) fixing and permeabilizing said cells;
- b) catalyzing the deposition of tyramide conjugated to a detectable label in cells comprising said intracellular analyte, whereby cells comprising said intracellular analyte are specifically labeled; and
- c) contacting said cells with a medium comprising a chaotropic agent to wash said cells;
- d) detecting a signal from cells comprising said detectable label using a flow cytometric device, wherein the presence of said signal is correlated to the presence of said intracellular analyte in said cells.

3. (Amended) The method of claim 1 or 2, wherein said signal is at least 20-fold greater than a signal obtainable by standard flow cytometry methods.

4. (Amended) The method of claim 1 or 2, wherein said signal is at least 50-fold greater than a signal obtainable by standard flow cytometry methods.

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5. (Amended) The method of claim 1 or 2, wherein said catalyzing step comprises:

- (i) incubating the fixed and permeabilized cells with a binding partner that specifically binds to said analyte, wherein said binding partner is conjugated to an enzyme that catalyzes the deposition of tyramide in the presence of tyramide and a substrate for said enzyme;
- (ii) removing unbound binding partner from said cells; and
- (iii) contacting bound binding partner with tyramide and said substrate for said enzyme, whereby said enzyme catalyzes the deposition of tyramide in said cells comprising said intracellular analyte.

6. (Amended) The method of claim 1 or 2, wherein said detectable label is a fluorochrome.

7. (Amended) The method of claim 6, wherein said fluorochrome comprises a fluorescent molecule selected from the group consisting of fluorescein, phycoerythrin, CY5, allophycocyanine, Texas Red, peridinin chlorophyll, and cyanine.

8. (Amended) The method of claim 5, wherein said enzyme is selected from the group consisting of hydrolases, peroxidases, oxidase, esterases, glycosidases and phosphatases.

9. (Amended) The method of claim 5 wherein said enzyme is horseradish peroxidase.

10. (Amended) The method of claim 1 or 2, wherein said catalyzing step comprises:

(i) incubating the fixed and permeabilized cells with a first binding partner that specifically binds to said analyte, and a second binding partner that specifically binds to said first binding partner, wherein said second binding partner comprises an enzyme, wherein said second binding partner is conjugated to an enzyme that catalyzes the deposition of tyramide in the presence of tyramide and a substrate for said enzyme the deposition of tyramide;

(ii) removing unbound second binding partner from said cells; and

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(iii) contacting bound second binding partner with tyramide and said substrate for said enzyme, whereby said enzyme catalyzes the deposition of tyramide in said cells comprising said intracellular analyte.

11. (Amended) The method of claim 10, wherein said second binding partner is an immunoglobulin-enzyme conjugate.

12. (Amended) The method of claim 1 or 2, wherein said one or more cells are one or more mammalian cells.

13. (Amended) The method of claim 12, wherein said one or more mammalian cells are selected from the group consisting of basal cells, epithelial cells, erythrocytes, platelets, lymphocytes, T-cells, B-cells, natural killer cells, granulocytes, monocytes, mast cells, Jurkat cells, neurocytes, neuroblasts, cytomegalic cells, dendritic cells, macrophages, blastomeres, endothelial cells, HeLa cells, tumor cells, interstitial cells, Kupffer cells, Langerhans' cells, Langerhans cells, littoral cells, tissue cells, adipose cells, CHO cells, KFL9, and K562 cells.

14. (Amended) The method of claim 1 or 2 wherein, said one or more cells are cultured cells.

15. (Amended) The method of claim 1 or 2, wherein said intracellular analyte is selected from the group consisting of intracellular cytokines, antigens, viral antigens, nuclear antigens, cytoplasmic antigens, organellar antigens, enzymes, cytoskeletal molecules, glycolipids, lipids, glycans, chaperones, RNA, DNA, messenger RNA, ribosomal RNA, signal transduction proteins, and structural proteins.

16. (Amended) The method of claim 1 or 2, wherein said intracellular analyte is not a natural component of said one or more cells.

17. (Amended) The method of claim 1 or 2, wherein said intracellular analyte cannot be detected by standard flow cytometry methods.

18. (Amended) The method of claim 1 or 2, wherein said one or more cells are obtained from a patient.

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19. (Amended) The method of claim 18, wherein said signal is correlated to a diagnosis of a disease in said patient.

20. (Amended) A kit for performing a method according to claims 1 or 2, wherein said kit comprises a medium comprising a chaotropic agent; an analyte-specific binding partner conjugated to an enzyme that catalyzes the deposition of tyramide in the presence of tyramide and a substrate for said enzyme; a substrate for said enzyme; and a tyramide reagent selected from the group consisting of unlabeled tyramide and tyramide conjugated to a detectable label, wherein if said tyramide reagent is unlabeled tyramide, said kit further comprises a tyramide-specific binding partner conjugated to a detectable label.

Please add the following new claim:

37. (New) The method of claim 1 or 2, wherein said signal is at least 10-fold greater than a signal obtainable by standard flow cytometry methods.

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Claims 1-36 are presently pending in the instant application, with claims 1-20 currently under examination. In the present submission, Applicant has cancelled claims 21-36, amended claims 1-20, and added new claim 37. The new and amended claims do not introduce new matter or require a new search. Rather, the amendments to the claims made herein merely clarify the subject matter of the claims using preferred terminology.

Notwithstanding the foregoing, Applicant expressly reserves the right to pursue subject matter no longer claimed in the instant application in one or more applications which may claim priority hereto. Applicant respectfully requests reconsideration of the claimed invention in view of the foregoing amendments and the following remarks.

Non Art-Related Remarks

Telephonic Interview

The courtesy extended to Applicant Dr. David Kaplan and Applicant's representative in the telephonic interview conducted by Examiner Gabel on January 30, 2003 is gratefully acknowledged and appreciated. The art-based rejections of the claims, and in particular the teachings of Roth et al., U.S. Patent No. 5,902,727, were discussed. Applicant's remarks in this regard are repeated below in the traversal of the art-based rejections.

35 U.S.C § 112, Second Paragraph

Applicants respectfully traverse in part the rejection of claims 1-20 under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the present invention.

When determining definiteness, the proper standard to be applied is "whether one skilled in the art would understand the bounds of the claim when read in the light of the specification." *Credle v. Bond*, 30 USPQ2d 1911, 1919 (Fed.Cir.1994). See also *Miles Laboratories, Inc. v. Shandon, Inc.*, 27 USPQ2d 1123, 1127 (Fed.Cir.1993) ("If the claims read in the light of the specification reasonably apprise those skilled in the art of the scope of the invention, § 112 demands no more.").

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Antecedent basis in claims 1, 2, and 5

Applicant respectfully submits that the foregoing amendments render the rejection of claims 1, 2, and 5 for an alleged lack of antecedent basis moot.

Omission of essential steps in claims 1 and 2

Applicant respectfully disagrees that claims 1 and 2 lack the essential element of correlating the signal detected from the claimed staining methods to the presence of the analyte of interest. Nevertheless, in an effort to advance prosecution, Applicant has amended claims 1 and 2 to explicitly indicate that the signal detected from cells labeled by the labeling method recited in the claims is correlated to the presence of the intracellular analyte in said cells.

Applicant submits that the amendments made in this regard do not further limit the claims, and should not be taken to do so. Applicant respectfully submits that the foregoing amendments render the rejection moot.

Antecedent basis in claims 3-20

Applicant respectfully disagrees that the indefinite article in the phrase "A method according to claim..." should be replaced with the definite article. The skilled artisan would be reasonably apprised that the phrase "A method according to claim..." in a dependent claim indicates that the claim refers back to and further limits the preceding claim, in accordance with 37 C.F.R. § 1.75. Applicants note that MPEP 608.01(n) specifically describes the use of the indefinite article in the context of dependent claims.

Nevertheless, in an effort to reduce issues and advance prosecution, Applicant has amended these claims, as suggested by the Examiner. Applicants note that the amendment is not intended in any way alter the scope of the claims, and should not be taken to do so. Applicant respectfully submits that the foregoing amendments render the rejection moot.

"Capable of" in claims 5 and 10

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Applicant respectfully submits that the foregoing amendments render the rejection of claims 5 and 10 for an alleged lack of antecedent basis moot.

Markush language in claim 15

Applicant respectfully traverses the rejection of claim 15 as allegedly being indefinite for its use of Markush language. The Examiner's statement that overlapping terms in the Markush group render the claim indefinite is not a sufficient basis for rejection of the language used by Applicant. *See, e.g.*, MPEP § 2173.05(h) ("the double inclusion of an element by members of a Markush group is not, by itself, sufficient basis for objection to or rejection of claims.... For example, the Markush group , "selected from the group consisting of amino, halogen, nitro, chloro, and alkyl" should be acceptable event though 'halogen' is generic to 'chloro'").

Moreover, the Examiner is incorrect that "'cytoplasmic antigens' and 'cytoskeletal molecules'... appear to be cell surface antigens." Paper No. 9, page 3. The skilled artisan would readily acknowledge that cytoplasm is contained within an intracellular space and that, therefore, the term "cytoplasmic molecules" refers to intracellular molecules. Similarly, the term "cytoskeleton" refers to an intracellular network of (actin-based) microfilaments, (tubulin-based) microtubules, and intermediate filaments. For the benefit of the Examiner in this regard, Applicants provide herewith pages 17 and 787 from *Molecular Biology of the Cell*, 3rd Ed. (1994), which discuss the fact that both the cytoplasm and the cytoskeleton are considered by those of skill in the art to comprise intracellular components.

Therefore, because the claims, when properly interpreted, meet the standards of 35 U.S.C. §112, second paragraph, Applicants respectfully request that the rejection be reconsidered and withdrawn.

Kit components in claim 20

Applicant has amended claim 20 to refer to specific components which may be included in the claimed kits for performing the methods of claim 1 and 2. Applicant respectfully submits that the foregoing amendments render the rejection moot.